

# **CRANIOFACIAL PHENOTYPE IN CUTIS LAXA**

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University of Pittsburgh, 2014

### **ABSTRACT**

Cutis laxa (CL) is a rare connective tissue disorder associated with mutations in extracellular matrix genes that leads to loose, redundant, lax skin and is often accompanied by cardiovascular, pulmonary, musculoskeletal or neurological complications. Literature suggests that individuals with CL have distinctive craniofacial features. Prior reports have been inconsistent and to date no quantitative, objective analysis of the craniofacial phenotype has been carried out on this population. To address this deficit, state-of-the-art 3-dimensional (3D) imaging was used to capture quantitative facial measurements in a sample of individuals with cutis laxa and compare the facial morphology of affected individuals to matched controls. The hypothesis that different forms of CL (acquired vs congenital) exhibit different craniofacial phenotypes was also tested. Subjects were recruited as part of the Genetics of Extracellular Matrix in Health and Disease Study and 3D images of the head and face were acquired using a 3DMD portable stereophotogrammetry system. Twenty-four facial landmarks were identified on each subject's 3D facial image, and from these landmarks, a set of standard anthropometric facial measurements was calculated. These shape coordinates were then subjected to principal components analysis to quantify and compare the major aspects of facial shape variation within and among groups. Congenital CL showed marked narrowing and

lengthening of the face and subjects with *ELN* mutations had the most severe facial phenotypes. The craniofacial shape did not correlate with the individual's skin elasticity supporting the conclusion that the craniofacial phenotype of CL is primarily caused by altered craniofacial development rather than skin laxity. The public health significance of this study is that craniofacial dysmorphism can occur as a part of many genetic syndromes. This type of quantitative study can be applied to any genetic disease with a distinct craniofacial phenotype and may assist the development of accurate, objective, sensitive and specific diagnostic criteria.

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## **PREFACE**

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## **1.0 INTRODUCTION**

### **1.1 CUTIS LAXA: SUB-TYPES AND CRANIOFACIAL FEATURES**

Cutis laxa is a rare connective tissue disorder characterized by generalized or localized lax skin, premature wrinkling or aging of the skin, redundant skin folds, cardiovascular issues, pulmonary complications, or musculoskeletal features. The true prevalence is not known but an estimate of 1:4,000,000 for all congenital types of cutis laxa has been reported in the Rhone-Alpes Eurocat Registry (E Robert, personal observation). Cutis laxa may be classified into two broader categories: congenital and acquired. Congenital cases have inherited or de novo mutations that cause cutis laxa visible at birth, whereas the acquired form is not fully understood; however, a life event occurs that causes cutis laxa later on in adulthood. It is a heterogeneous condition that may be divided into many different subtypes (Berk et al., 2012).

Several reports have described distinct craniofacial features in CL populations. These reports have generally been “qualitative” in nature and a systemic assessment of the craniofacial phenotype has been lacking. Below we will summarize the different forms of cutis laxa, focusing on their etiology, and what, if anything is known about their craniofacial phenotype. It is important to characterize the craniofacial phenotype for cutis laxa because it is a rare condition that few healthcare professionals will encounter during his/her career. As a result, this condition may not be recognized through a physical exam and consequently not be diagnosed. This study

could be the foundation of characterizing and quantifying the facial features of CL to make the phenotype well known and be used for diagnostic purposes in the future.

### **1.1.1 Autosomal Dominant Cutis Laxa (ADCL)**

ADCL is caused by mutations in the elastin (*ELN*) gene, which codes for a protein called tropoelastin. The tropoelastin protein forms elastin, which is a mature protein polymer and builds an individual's elastic fibers. The mutation was first identified by Tassabehji et al (1998). This study found a novel frameshift mutation and performed EM (electron microscopy) suggesting the mutant tropoelastin protein changes formation of elastin fibers once incorporated in the matrix (Tassabehji et al., 1998). Another study built on this and discovered additional *ELN* mutations in ADCL (Zhang et al., 1999).

Because elastic fibers give structural support to the skin, heart, lungs, and blood vessels, individuals with autosomal dominant cutis laxa may have lax skin, cardiovascular concerns such as aortic root dilatation, pulmonary complications like emphysema, and inguinal hernias (Callewaert et al., 2011; He et al. 2012). Individuals with an *ELN* mutation have been described as having a long face, large pliant ears, and a long philtrum. Individuals may also have a beaked nose and blepharochalasis or eyelid inflammation and ptosis or drooping of the upper eyelid often occurs (Hadj-Rabia et al., 2013). Autosomal dominant cutis laxa causes patients to have a prematurely aged appearance of the face (Uitto et al., 2013). They have also been described as having a coarse face with bitemporal narrowness, low nasal bridge, flat broad nose, periorbital fullness, full cheeks, high palate, general skin laxity especially in the face and buttocks, and lax abdominal musculature (Graul-Neumann et al., 2007). Usually, the skin's appearance is the

focus and reported as an aged appearance with loose redundant skin folds of the cheeks and chin, a high forehead, a long philtrum and large ear lobes (Callewaert et al., 2011).

### **1.1.2 Autosomal Recessive Cutis Laxa 1A (ARCL1A)**

ARCL1A is caused by mutations in the fibulin-5 (*FBLN5*) gene. A homozygous missense mutation was first reported to cause this type of cutis laxa. When analyzing individuals from a large family, the mutation segregated with this disease (Loeys et al., 2002). The *FBLN5* gene codes for a protein called fibulin-5 that assists with the assembly of elastic fibers and in adults can be found in skin, aorta, lung, and uterus (Yanagisawa et al., 2002; Kowal et al., 1999). Fibroblast studies verified the protein's role in creating elastic fibers, and confirmed the significance of pathogenic, missense mutations that have a loss of function effect (Hu et al., 2006; Claus et al., 2008). Individuals with autosomal recessive cutis laxa 1A may have loose skin, hernias, developmental emphysema and supravalvular aortic stenosis (Loeys et al., 2002). Phenotypic facial features have been reported such as an aged appearance of the skin including the face, bilateral ptosis, drooping cheeks, large ears, and a small mouth (Claus et al., 2008).

### **1.1.3 Autosomal recessive cutis laxa 1B (ARCL1B)**

ARCL1B is caused by mutations in the fibulin-4 (*FBLN4*) gene, which codes for a protein component of the elastic fibers. A homozygous missense mutation was identified and the effects of the mutation were explored using fibroblasts and analyzing mRNA expression. A decrease of fibulin-4 protein was found in the extracellular matrix of the cultured fibroblasts (Huchtagowder et al., 2006). Studies looking at compound heterozygous mutations found a lack of fibulin-4 in

the extracellular matrix of cultured fibroblasts. As a result of these mutations, an unstable mutant protein and an unstable mRNA were produced (Dasouki et al., 2007). Skin fibroblasts from individuals with *FBLN4* mutations show increased activity of the TGF $\beta$  signaling pathway (Renard et al., 2010). Kappanayil et al. (2012) conducted a study with 22 subjects and identified two mutations in fibulin-4. Individuals with autosomal recessive cutis laxa 1B can have a severe phenotype that includes lax skin, aneurysms of the aorta, arterial tortuosity, inguinal hernias, hypermobility, and emphysema. Case reports have shown a homozygous premature stop codon, which yields multiple bone fractures, tortuous arteries, and generalized cutis laxa (Erickson et al., 2012). Craniofacial features that have been reported include loose, flabby cheeks and hypoplasia of the lower jaw, a hooked nose, small palpebral fissures and dysplastic ears (Sawyer et al., 2012; Hoyer et al., 2009).

#### **1.1.4 Autosomal recessive cutis laxa 1C (ARCL1C)**

ARCL1C is caused by mutations in the latent transforming growth factor beta binding protein 4 (*LTBP4*) gene. Defects in this gene cause lax skin, severe emphysema, gastrointestinal, genitourinary, and musculoskeletal issues. Premature stop codon mutations are frequently found in *LTBP4* causing greatly reduced mRNA expression. In *LTBP4* deficient tissues and cell cultures the elastic fibers are disorganized and the amount of TGF- $\beta$  released from the extracellular matrix is increased (Urban et al., 2009). Individuals with autosomal recessive cutis laxa 1C have been reported to have a sloping forehead, sparse hair on the temporal sides, large ears, hypertelorism, a low nasal bridge, a beaked nose, sagging cheeks, and retrognathia (Callewaert et al., 2012).

### **1.1.5 Autosomal recessive cutis laxa 2A (ARCL2A)**

ARCL2A is caused by mutations in the gene for the  $\alpha 2$  subunit of the lysosomal proton transporter ATPase, (*ATP6V0A2*), which codes for a component of a protein complex responsible for secretory vesicle acidification. Mutations in *ATP6V0A2* result in a loss of function and abnormal organization of the Golgi apparatus. Patients with ARCL2A also have altered glycosylation of serum proteins (Kornak et al., 2008). Individuals with autosomal recessive cutis laxa 2A may have features including lax skin, hip dislocations, hernias, myopia, severe developmental delay, and seizures (Huchtagowder et al., 2009). Their craniofacial phenotype has been described as a triangular-shaped face, downslanting palpebral fissures and a broad nasal root (Fischer et al., 2012).

### **1.1.6 Autosomal recessive cutis laxa 2B (ARCL2B)**

ARCL2B is caused by mutations in the gene for pyrroline-5-carboxylate reductase 1 (*PYCR1*), a mitochondrial enzyme necessary for proline biosynthesis (Reversade et al., 2009). Reversade et al. in 2009 found highest levels of *PYCR1* expression in the skin and bone and mutations caused a loss of function of the protein. Guernsey et al. (2009) identified a missense mutation in the *PYCR1* gene that led to exon skipping. A further study gathered 33 individuals from 27 families to examine the clinical features and found that hypotonia, IUGR (intrauterine growth retardation), and psychomotor retardation coupled with certain facial features could provide clues for a potential diagnosis (Dimopoulou et al., 2013). An individual may have features including lax skin, growth delay, joint and skeletal problems, developmental delay, and microcephaly. There are reports of lax face, broad forehead, midface retrusion, prognathism, and

large protruding ears (Yildirim et al., 2011). Another study mentioned a triangular face, blue sclerae, prominent ears, and microcephaly while another study reported prominent forehead, protruding ears, myopia, and esotropia (Kouwenberg et al., 2011; Lin et al., 2011).

### **1.1.7 Autosomal recessive cutis laxa 3 or De Barsy syndrome (ARCL3)**

ARCL3 is caused by mutations in the aldehyde dehydrogenase 18 family, member A1 (*ALDH18A1*) gene, which codes for a protein required for the biosynthesis of proline, ornithine and arginine. Bicknell and co-workers studied a homozygous missense mutation, p.H784Y, predicted to be deleterious by the SIFT software. The effect of this mutation in *ALDH18A1* is not currently understood (Bicknell et al., 2008). Features of ARCL3 include lax skin, growth retardation, moderate to severe intellectual disability, cataracts, and joint laxity. The craniofacial phenotype in ARCL3 has been described as a high forehead with frontal bossing, depressed nasal bridge, short upturned nose with hypoplastic nasal alae, and thin lips as well as deep set eyes, corneal clouding, prominent cheek jowls, high-arched palate, and micrognathia (Skidmore et al., 2011).

### **1.1.8 MACS syndrome**

MACS syndrome was named after its major manifestations including macrocephaly, alopecia, cutis laxa, and scoliosis (Basel-Vanagaite et al., 2009). Therefore, an individual may have features including lax skin, macrocephaly, alopecia, and scoliosis, and joint laxity. This form of cutis laxa is caused by mutations in the Ras and Rab interactor 2 (*RIN2*) gene (Basel-Vanagaite et al., 2009). For example, a homozygous frameshift mutation was identified causing RIN2



protein production to be reduced (Basel-Vanagaite et al., 2009). Syx et al. (2010) identified a *RIN2* mutation, which caused a premature stop codon and as a result, nonsense-mediated decay of the mRNA. However, when reviewing the clinical findings of these patients in this study, the authors thought that three individuals did not exhibit cutis laxa of the skin. They proposed that MACS syndrome may not be the most appropriate name and argued that this condition is more similar to Ehlers-Danlos syndrome (Syx et al., 2010). Thus, the precise classification of *RIN2*/MACS syndrome remains unclear. Studies have commented on the craniofacial phenotype being facial coarsening and macrocephaly, downslanting palpebral fissures, puffy droopy eyelids, and full lips (Basel-Vanagaite et al., 2009; Syx et al., 2010).

#### **1.1.9 X-linked recessive form: occipital horn syndrome (OHS)**

This form of CL is caused by mutations on the X chromosome and is a milder variant of Menkes disease, which is a defect of copper metabolism. In OHS, low ceruloplasmin and serum copper levels may be present. This disorder is caused by mutations in the *ATP7A* gene, encoding a copper transporter.. Studies in the early 1990s analyzed a splice mutation that was thought to yield a milder phenotype than Menkes. The amount of mRNA was found to be reduced but not completely absent. A possible explanation was that some level of normal splicing was occurring allowing for some remaining protein function (Kaler et al., 1994). Further investigation of OHS confirmed that splicing was involved and the disorder can be considered a milder variant of Menkes disease (Das et al., 1995). Two novel missense mutations were reported within individuals with distal motor neuropathy in the absence of obvious copper deficiency (Kennerson et al., 2010). One study reviewed mutations previously reported in the literature (Tumer, 2013). Individuals who have OHS may have hernias, skeletal abnormalities, joint laxity, and bladder

diverticula. The most notable feature is the occipital horns, which may become more apparent as these individuals age. The craniofacial phenotype is not well described but reports of kinky hair have been made for Menkes patients while OHS patients have occipital bony abnormalities, dolichocephaly, prominent ears, downslanting palpebral fissures, and ptosis (Tumer, 2013).

#### **1.1.10 Arterial tortuosity syndrome (ATS)**

ATS is caused by mutations in the solute carrier family 2 (facilitated glucose transporter), member 10 (*SLC2A10*) gene, which codes for a protein called GLUT10, involved in bone and blood vessel development (Coucke et al., 2006). An individual with arterial tortuosity syndrome may have features including tortuous arteries with an increased risk of aneurysms and stenosis. The craniofacial phenotype is described as having acrogeria, a long face, sagging cheeks, thin nose with hypoplastic alae nasi, bilateral lower blepharochalasia and mild blepharophimosis (Castori et al., 2012).

#### **1.1.11 Craniofacial phenotype in Cutis Laxa**

There are many shared facial features among these subtypes of cutis laxa. The majority demonstrate an aged appearance due to premature wrinkling and sagging skin. However, these features are difficult to characterize due to the lack of quantitative analysis. The current qualitative reports are subjective in nature as individuals employ different descriptive terms that overlap. For example, drooping cheeks are the most common feature seen in ADCL, ARCL1A, ARCL1B, ARCL1C, ARCL3, and ATS but the terms flabby, sagging, and full are all utilized to describe the same feature (Graul-Neumann et al., 2007; Claus et al., 2008; Sawyer et al., 2012;

Hoyer et al., 2009; Callewaert et al., 2012; Skidmore et al., 2011; Castori et al., 2012). Other common features include a long face in ADCL and ATS, prominent ears in ADCL, ARCL1A, ARCL1C, ARCL2B, and OHS, and ptosis in subtypes ADCL, ARCL1A, MACS syndrome, and OHS (Hadj-Rabia et al., 2013; Claus et al., 2008; Callewaert et al., 2012; Yildirim et al., 2011; Tumer, 2013). Additional shared features include a hooked nose for subtypes ADCL, ARCL1B and ARCL1C, triangular-shaped face in ARCL2A and ARCL2B, and frontal bossing in ARCL2B and ARCL3 (Table 1) (Hadj-Rabia et al., 2013; Sawyer et al., 2012; Hoyer et al., 2009; Callewaert et al., 2012; Fischer et al., 2012; Kouwenberg et al., 2011; Lin et al., 2011; Skidmore et al., 2011). However, depending on the expertise of the person reporting the features, some may be overlooked or remain unreported. Furthermore, some craniofacial characteristics may not appear abnormal but still may be distinct from controls in quantitative analysis, and it is these features that could be missed by case reports. It is important to review the various phenotypes because although there are some shared features among them, each subtype has a different craniofacial phenotype, which can be quantified. If this is well characterized, genotype-phenotype correlations will be better understood and healthcare professionals could not only diagnosis CL patients but predict what mutation the individual should have before undergoing genetic testing.

**Table 1.** Subtypes of CL, causative gene, and craniofacial features

Type	Gene	Features
ADCL	ELN	Aged appearance, long philtrum and face, large ears
ARCL1A	FBLN5	Aged appearance, bilateral ptosis, drooping cheeks, large ears, small mouth
ARCL1B	FBLN4	Flabby cheeks, lower jaw hypoplasia, hooked nose, small palpebral fissures, dysplastic ears
ARCL1C	LTBP4	Sloping forehead, sparse hair, large ears, hypertelorism, low nasal bridge, beaked nose, sagging cheeks, retrognathia
ARCL2A	ATP6V0A2	Triangular-shaped face, downslanting palpebral fissures, broad nasal root
ARCL2B	PYCR1	Lax, triangular face, broad forehead, midface retrusion, prognathism, large protruding ears, myopia, and esotropia
ARCL3	ALDH18A1	Frontal bossing, depressed nasal bridge, short upturned nose with hypoplastic nasal alae, thin lips, saggy cheeks, and micrognathia
MACS syndrome	RIN2	Facial coarsening, macrocephaly, downslanting palpebral fissures, puffy, droopy eyelids, and full, everted lips
Occipital Horn Syndrome (OHS)	ATP7A	Occipital bony abnormalities, dolichocephaly, prominent ears, downslanting palpebral fissures, ptosis

## **1.2 CRANIOFACIAL PHENOTYPES IN OTHER CONNECTIVE TISSUE DISORDERS**

### **1.2.1 Williams-Beuren syndrome (WBS)**

This condition is caused by a deletion of 7q11.23 (Ewart et al., 1993). Both *ELN* and *GTF2IRD1* are within the deleted region. The craniofacial phenotype of WBS is characterized by bitemporal narrowing, a broad forehead, flattened malar, puffy appearance of the periorbital region, wide mouth and nasal tip, long philtrum, full cheeks, and micrognathia (Ewart et al., 1993). Homozygous deletion of *GTF2IRD1* results in face abnormalities in mice, similar to the craniofacial phenotype for people with WBS. *GTF2IRD1* controls expression of 2 genes that are involved in bone and craniofacial development: *Gsc* (goosecoid) and *Hoxc8* (Tassabehji et al., 2005). However, the contribution of *ELN* haploinsufficiency, if any, to the WBS craniofacial phenotype remains to be determined.

### **1.2.2 Marfan syndrome (MFS)**

MFS is caused by mutations in fibrillin-1 gene (*FBNI*) (Kainulainen et al., 1990; Sood et al., 1996). A morphometric analysis of MFS patients identified retrognathia, high palate, and dental issues such as root deformities (De Coster et al., 2004). Motohashi created a system where certain diseases associated with dysmorphology would have the ability to be studied quantitatively using a schematic diagram to take measurements of the head (Motohashi, 1985). How to specifically study and quantify craniofacial growth in this population was also investigated (Grewe et al., 1971). Craniofacial abnormalities in Marfan patients were studied in

a group of 15 adults and found to be prevalent within this population and also an association with obstructive sleep apnea was discovered (Cistulli et al., 2001). When the specific abnormalities were quantified, the palate was found to be high and narrow and the lower jaw retrognathic (Wrestling et al., 1998; Poole, 1989). Further studies confirmed that the palate in patients with Marfan syndrome was larger and taller than individuals without this condition. A characteristic long face was also found to be associated with this connective tissue disorder (De Coster et al., 2004).

### **1.3 QUANTITATIVE ANALYSIS OF CRANIOFACIAL FEATURES**

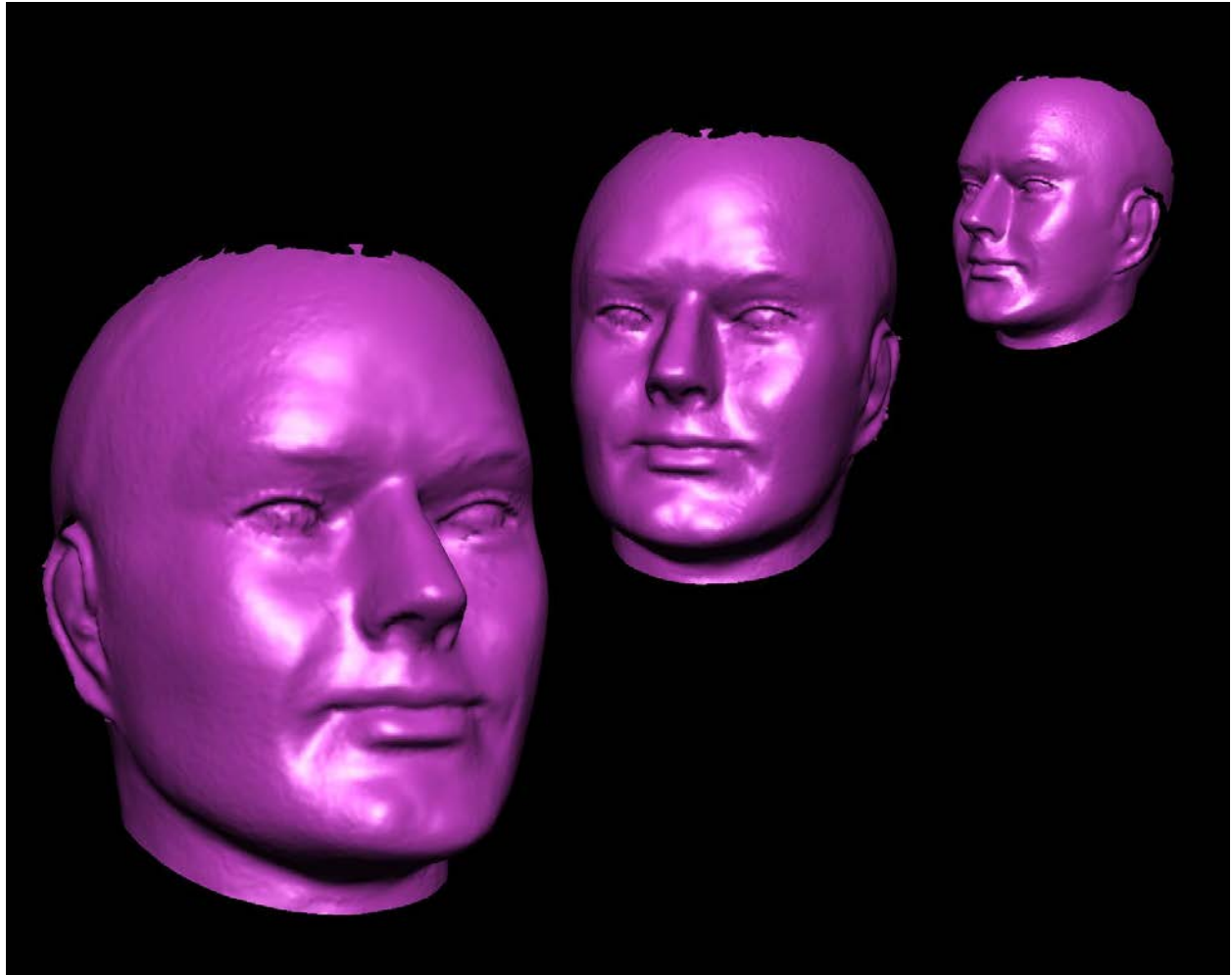
#### **1.3.1 Direct anthropometry**

Traditionally, handheld calipers have been used to measure the craniofacial complex (Farkas, 1994). This is considered direct anthropometry because the measurement method requires direct contact with the subject's head and face. While highly reliable and inexpensive, this approach to measurement has several drawbacks. It can be invasive and very difficult to perform on uncooperative subjects. It can also be quite time consuming, requiring patients to remain still for long periods of time (Wong et al., 2008).

#### **1.3.2 Digital 3D stereophotogrammetry**

Stereophotogrammetry involves quantifying objects from photographs. In recent years, 3D stereophotogrammetry has become a popular technique for capturing the geometry of the facial

surface (Heike et al., 2010). Based on relatively simple principles of triangulation, 3D stereophotogrammetry is a non-invasive acquisition method capable of very fast captures (less than 1 second). Commercially available systems allow users to capture photorealistic and geometrically accurate 3D maps of the human face and provide software allowing users to manipulate and quantify the resulting surfaces. Compared with direct anthropometry, this method is preferred when conducting routine facial imaging as it reduces the amount of time required for collecting the data and allows for numerous quantification methods following capture (Artopoulos et al., 2013; Maal et al., 2010). Additionally, digital 3D stereophotogrammetry is desirable because it is easily reproduced (Maal et al., 2011); this technology has been studied and validated by numerous independent studies (Weinberg et al., 2004; 2006; Aldridge et al., 2005; Losken et al., 2005; Wong et al., 2008; Heike et al. 2009).



**Figure 1.** An example of a 3D model without texture

#### **1.4 SPECIFIC AIMS**

If individuals with cutis laxa exhibit distinctive craniofacial features, then it should be possible to quantify them. To date, no studies have focused specifically on the cutis laxa craniofacial phenotype. In the current literature, facial features are only briefly mentioned with inconsistent qualitative descriptions. To address this deficit, this study will use modern phenotyping methods (3D imaging and advanced morphometric methods) to provide a more complete understanding of



the craniofacial complex in cutis laxa. An improved understanding may offer additional insights into the phenotypic similarities and differences among subtypes of cutis laxa and the possible role of extracellular matrix genes and proteins in craniofacial development. For this study, we hypothesized that different forms of CL will present distinct craniofacial phenotypes suggesting possible genotype-phenotype correlations. The following specific aims were proposed:

1. Conduct a quantitative evaluation of facial shape in a cohort of individuals with CL using a portable 3D stereophotogrammetry system.
2. Compare the craniofacial shape of CL patients to matched controls using a geometric morphometric approach.
3. Investigate if craniofacial features correlate with the type of CL, the specific gene defect involved or with objective measures of skin laxity.

## **2.0 MATERIALS AND METHODS**

Institutional Review Board (IRB) approval for this study was obtained at the University of Pittsburgh, and the approval letter may be found in Appendix A. The Genetics of Extracellular Matrix in Health and Disease is currently funded through the NIH.

### **2.1 CLINIC PROTOCOL**

#### **2.1.1 Recruitment**

Participants were either self-referred or referred by physicians and/or genetic counselors who contacted the research team. Additional participants were recruited through an online support group or through relatives of a family already enrolled into the research study. Once all the necessary contact information was gathered and the individual had a clinical or probable diagnosis of cutis laxa or had a first degree relative with a clinical or probable diagnosis, a screening process occurred to determine eligibility. This procedure was conducted over the telephone with the participant where he/she was asked a series of questions about his/her skin or his/her first degree relative's skin: does he/she have loose, lax skin, skin in redundant folds, inelastic or doughy skin, premature aging of the skin, or excessive premature wrinkling. If an individual answered "yes" to at least three of these questions, then he/she was eligible for

participation in the research study. If he/she did not answer “yes” to at least three of these questions, then additional questions about family member’s other organ systems were asked: any arterial tortuosity, aneurysms, emphysema, spinal disease, diverticula, or hernias. If the subject answered “yes” to at least one of these questions then he/she was eligible. The next step was gathering demographic and diagnosis-related information from the subject including confirming participant’s contact information, age of onset for the cutis laxa, and if a diagnosis was made, name and contact information of the physician who made the diagnosis.

### **2.1.2 Informed Consent**

Once the participant was determined to be eligible, informed consent was obtained. This involved reviewing and explaining each section of the consent form (describing the purpose of the study and the research clinic) with the participant and answering any questions. If the participant attended a research clinic through the University of Pittsburgh Medical Center, then the individual was re-consented (including reviewing all research testing that would be performed) in person before any research activities were carried out. The participant and the person obtaining consent signed the consent form before any research activities were performed.

### **2.1.3 Research Activities**

Any activity that was completed depended upon whether the subject was participating remotely via his/her physician or attending a research clinic in Pittsburgh. However, all facial imaging was completed in Pittsburgh.

### **2.1.3.1 Affected Individuals' Participation through Research Clinics**

When an individual attended a cutis laxa research clinic in Pittsburgh, activities included a cutis laxa questionnaire which covered personal medical history and pedigree, blood (or, less often, saliva) sample and vitals (measuring height, weight, and blood pressure), genetics evaluation including two skin punch biopsies, craniofacial imaging, skin and vascular elasticity testing, a private meeting with the principal investigator, lymphedema measurements, pulmonary function test (PFT) and chest computed tomography (CT), respiratory questionnaires, echocardiogram, and DEXA scan. All of these tests were performed at the Clinical and Translational Research Center in Montefiore Hospital with exception of the chest CTs, which were completed at the Hillman Cancer Center.

Early in the clinic visit participants' vitals (height, weight, and blood pressure) were taken and his/her blood drawn for DNA, plasma, and serum isolation. In some cases, a saliva sample was also collected for DNA isolation (if blood could not be taken). The genetics evaluation was carried out by trained genetics personnel and consisted of a physical exam. The photographs were obtained with permission and the cutis laxa questionnaire was filled out (see Appendix B). This questionnaire obtained the pedigree, personal medical history, and review of systems. After the exam, a skin biopsy was obtained from participants who were willing and cells were not already available from a previous biopsy. Biopsies were used for histology, electron microscopy (EM), and growing fibroblasts.

Craniofacial imaging was performed using a 3dMDface digital 3D stereophotogrammetry system (Atlanta, GA). This testing was done on any affected individual who could remain still for at least five minutes with the youngest participant being 2 years old and is described in greater detail in section 2.3.

Skin elasticity testing consisted of using a DermaLab skin elasticity module that measured how elastic the skin was (elastic modulus, viscoelastic modulus, and retraction time). This testing was performed on all affected individuals who could remain still for at least 10 minutes.

#### **2.1.3.2 First-Degree Relatives' Participation**

Unless these relatives were known heterozygous carriers for a recessive mutation, for example, in *LTBP4*, participation involved receiving informed consent and giving a blood or saliva sample in order to complete genetic testing and confirm the results of the proband or to compare when performing whole exome sequencing. If the individuals were carriers, the participants received the same testing as individuals with cutis laxa.

## **2.2 LABORATORY PROTOCOLS**

### **2.2.1 Mutation Analysis**

DNA was isolated for each participant for mutation analysis. This analysis was completed usually from peripheral blood, or in some cases on saliva or cultured fibroblasts from a skin biopsy. Isolation was achieved utilizing phenol chloroform extraction or the PureGene System (Qiagen) for blood samples, and the Oragene protocol (DNA Genotek) for saliva samples. DNA concentration for the preparation was measured by UV spectrophotometry. Working solutions were prepared at a standard concentration of 50 ng/μl. Target gene sequences of *ELN*, *FBLN4*, *FBLN5*, *LTBP4*, *ATP6V0A2*, or *PYCR1* which included exons and flanking parts of introns were

amplified by PCR. ExoSAP-IT and ABI BigDye Terminator Kit (Applied Biosystems) were employed for cleaning the PCR product and for the sequencing reaction, respectively. Sense and antisense strand sequencing was completed using the University of Pittsburgh Genomics and Proteomics core facility. Sequencher (Gene Codes Corporation) and the UCSC Genome Browser were employed for analysis. Mutations were confirmed using separate amplification products and, if necessary, through RNA studies.

## **2.3 3D FACIAL IMAGING**

### **2.3.1 The Participants**

33 subjects with confirmed or suspected cutis laxa ranging in age from 2-70 years were recruited as part of the Genetics of Extracellular Matrix in Health and Disease Study at the University of Pittsburgh and attended a cutis laxa research clinic.

### **2.3.2 The Imaging Process**

As part of the research testing, 3D images of the head and face were obtained using a 3dMDface portable stereophotogrammetry system. The unit consists of six medical grade digital cameras with overlapping fields of view that simultaneously capture the face. Almost concurrently a high resolution color image of the face is acquired. These images are fed into a laptop with image processing software that automatically aligns the various images into a complete 3D model. The result is a dense 3D point cloud representing the complete 180-degree surface geometry of the

human face. To enhance visualization, this point cloud is then connected into a 3D mesh. This mesh is then filled in to create an airtight 3D surface and the pixels from the color images are mapped onto the geometry to give the 3D facial surface lifelike skin texture and color. The entire acquisition process takes place in less than 4 milliseconds. The post-acquisition processing takes an additional 20 seconds.

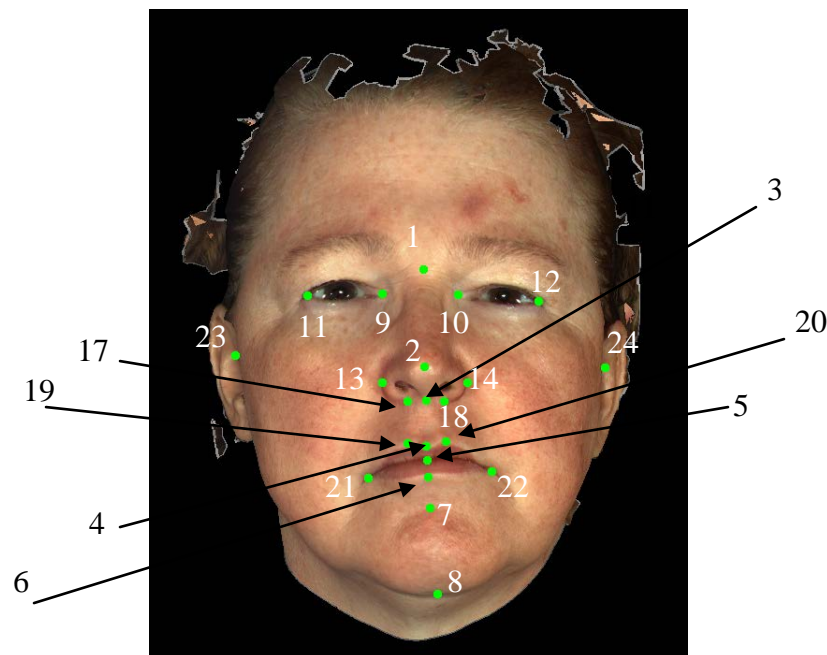
Great care was taken in preparing participants for the 3D photographs by positioning them in the ideal position with respect to the camera and removing any hair that was obstructing the face with a headband and/or clips. Participants were told to look at the camera straight on, remain still, and do not smile. After the image was taken, it was reviewed for quality and a second image was taken if necessary (if the participant had moved, mouth was open, etc.). All imaging was performed by trained personnel. The images were then transferred to the 3D Imaging and Morphometrics Lab within the Center for Craniofacial and Dental Genetics, School of Dental Medicine.

### **2.3.3 Landmarking**

The subjects' 3D images were "cleaned" by removing any unnecessary portions of the facial images such as a subject's shirt, bottom of neck, or sections of hair using the 3dMD-Patient software package. Using 3dMDVultus software, 24 standard anatomical landmarks were then identified on each subject's 3D facial image: these included the (1) nasion, (2) pronasale, (3) subnasale, (4) labiale superus, (5) stomion, (6) labiale inferus, (7) sublabiale, (8) gnathion, (9, 10) endocanthion (right and left), (11, 12) exocanthion (right and left), (13, 14) alare (right and left), (15, 16) alar curvature point (right and left), (17, 18) subalare (right and left), (19, 20) crista philtri (right and left), (21, 22) chelion (right and left), and (23, 24) tragion (right and left). The

coordinate positions (x,y,z) associated with the landmarks were then saved as text files for later analysis.

After each 3D image was landmarked, the placement of every landmark on all subjects' facial images was verified by a second rater for quality control. As an additional quality control measure, all raters undergo training prior to landmarking. This involves collecting the full set of 24 landmarks twice on an independent set of 20 subjects and evaluating the intraobserver error of the landmark placement. Intraclass correlation coefficients of .90 or greater must be achieved for every landmark in each of the three principal coordinate axes (x,y,z) in order to proceed with data collection. As another precaution after the landmarks had been placed on each subject, a 'Landmark Collection Form' was completed, which is a fillable pdf form used to create notes about missing regions, reasons why a landmark was not placed, or other details that would assist another person in understanding the choices an individual made while landmarking.



**Figure 2.** Landmarked facial image (15, 16 cannot be shown on this 2D surface)



### 2.3.4 Statistical Analysis

Following landmarking, the cutis laxa dataset was reviewed and individuals who met any exclusion criteria were removed from the sample. The exclusion criteria were the following for the cutis laxa cases: history of major facial surgeries, non-Caucasian ancestry (because non-Caucasian controls did not exist in the database), missing data due to poor 3D image, unaffected carriers of a recessive form of cutis laxa, and individuals who did not have a definitive diagnosis of cutis laxa. Based on these criteria the sample size was reduced from 33 to 10 subjects: five congenital cases of cutis laxa and five acquired cases of cutis laxa. 8 individuals were removed due to ancestry, 5 participants were removed for a history of major facial surgeries (including rhinoplasty, multiple facelifts, jaw and chin surgery, and multiple injections and fillers), 5 did not have a clinical diagnosis of cutis laxa, 3 individuals were unaffected carriers, 1 individual had missing data due to a poor 3D image, and 1 participant was an outlier and was removed from the sample set. The next step in this process was to compare these cases to demographically-matched controls obtained through the 3D Facial Norms Project (PI: Seth Weinberg), a database of 3D facial images, landmarks, and measurements comprised of over 2400 healthy individuals between age 3 and 40. These controls also had their own set of exclusion criteria: history of significant facial trauma or facial surgery, personal or family history of a craniofacial syndrome or birth defects, missing data due to poor 3D image, and non-Caucasian ancestry. Based on these criteria, four controls matched on age, sex, and ethnicity to each cutis laxa case were identified for a total sample size of 50: 10 cases and 40 controls. A t-test confirmed that the ages did not differ between the two groups (see Table 2 in Results).

A geometric morphometric analysis was then carried out on the landmark coordinate data. Briefly, the 3D facial landmark coordinates for all 50 subjects were registered to one

another using the Procrustes superimposition method. The process removes scaling effects from the coordinate data and ensures that all of the subjects are located within the same coordinate space. The newly transformed coordinates represent shape. Principal component analysis (PCA) was then applied to the shape coordinates in order to summarize the major modes of shape variation (shape components) present within the entire dataset. By plotting the scores on each extracted principal component against one another, it is possible to identify those components capable of distinguishing groups. As a follow-up analysis, a subset of seven landmarks comprising the nose and philtrum was subjected to canonical variates analysis (CVA). This approach looks for the best combination of variables (in this case shape coordinates) that discriminate across groups most efficiently. Both PCA and CVA are multivariate data reduction approaches.

One of the advantages of geometric morphometrics is that the data used in the analysis (x,y,z coordinates) contain intrinsic geometric qualities. Because of this, the shape variation uncovered by the PCA and CVA can be modeled as deformations of the average shape. This means that once a PC or CV is determined to be important for distinguishing between the groups of interest, the associated shape changes involved can be visualized. This can be done using wireframes for simple visualization or surface warps for more intuitive perspective. In the present study, the program MorphoJ was used for all geometric morphometric analyses and the program Landmark was used to generate the surface warps based on the PCA and CVA results.

To determine whether an association existed between a patient's skin elasticity and craniofacial shape in the cutis laxa sample, Pearson correlation coefficients were calculated. This can measure the dependence between two variables and then determining p values can evaluate statistical significance. The specific components of skin elasticity selected were

viscoelastic modulus (VE), elastic modulus (E), and retraction time (RT). V and E are measures of how easy it is to deform the skin and RT is the amount of time it takes the skin to return to its original position. D is a composite variable that differentiates controls from cases the best. The formula for D is:  $-27.570 + 0.187 \times \text{Age} + 2.795 \times \text{VE} + 1.267 \times \text{E}$ . These measures of skin elasticity were compared to the scores on any PCs or CVs that showed strong evidence of separating cutis laxa cases from controls.

### 3.0 RESULTS

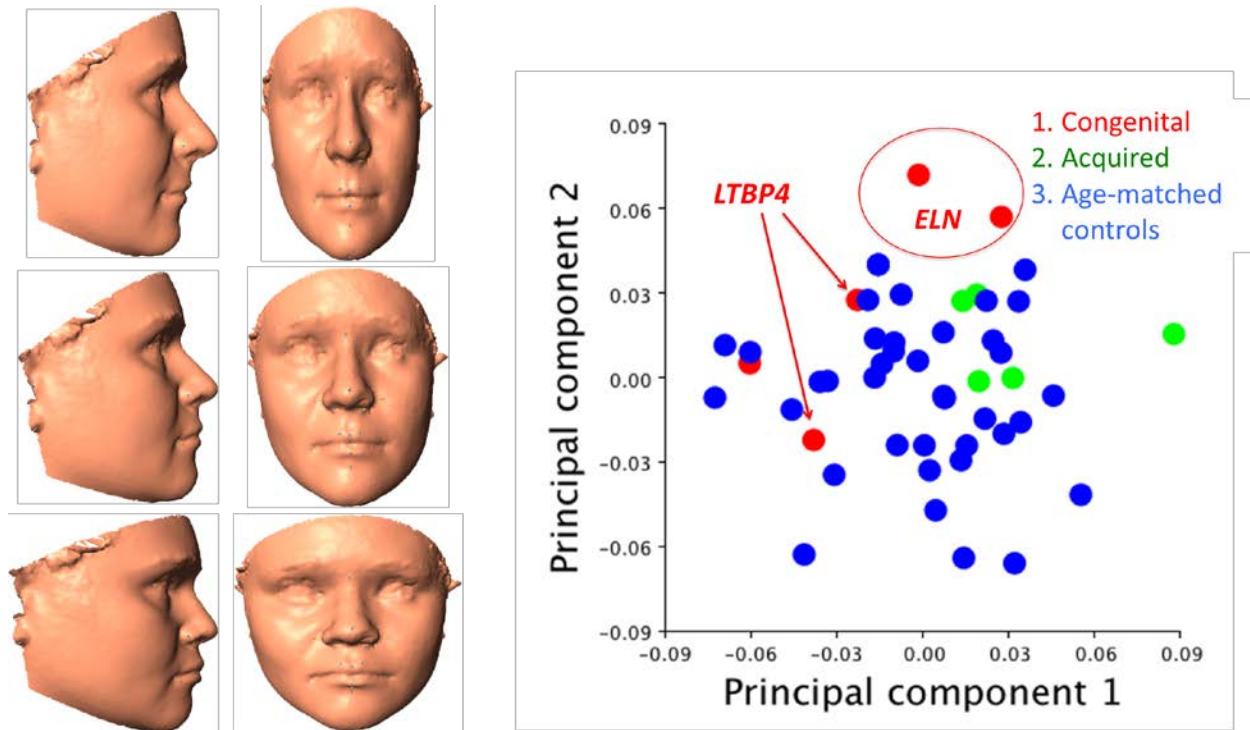
After applying the exclusion criteria, 10 individuals remained: 5 congenital cutis laxa cases (2 with ELN mutations, 2 with LTB4 mutations and 1 with an unknown mutation) and 5 acquired cutis laxa cases. A t-test confirmed that the ages did not differ significantly between cases and controls (Table 2).

**Table 2.** Sample size and age

Group	N	Age (years)	SD	P value
Cases	10	35.9	20.1	0.991
Controls	40	35.8	19.3	

N: sample size, Age: mean age, SD: standard deviation.

The first principal component (PC1) explained 22.7% of the facial shape variation. PC1 appears to show congenital cases separating from the acquired cutis laxa cases (Figure 3). However age was the primary driver of shape variation in this component; acquired cases tend to be on the older end of the age spectrum. PC2 explained 18.2% of facial shape variation. PC2 showed acquired cases and controls clustering together while the congenital cases separated primarily from controls (Figure 3). To confirm this pattern, the mean scores on PC2 were compared across the three groups with ANOVA - using Bonferonni pairwise comparisons (Table 3); congenital cases significantly differed from controls ( $p = 0.016$ ).



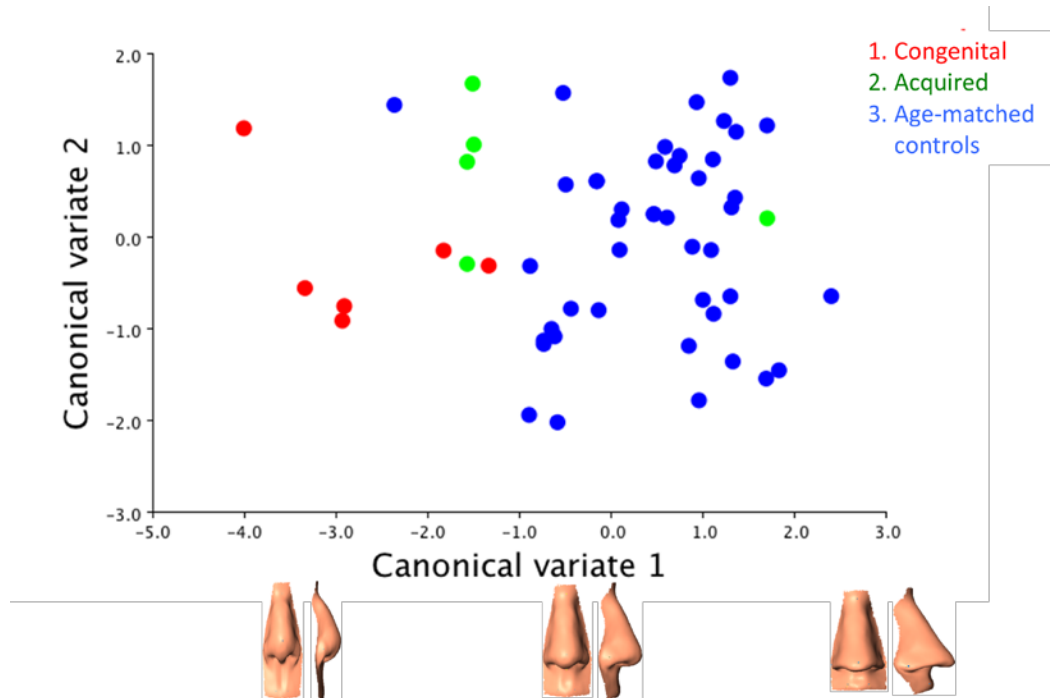
**Figure 3. PCA plot and facial warps showing the variation associated with PC2.** The facial shape associated with congenital CL is represented by the top face. The control facial shape is represented by the bottom face. Individuals with known mutations are identified by a circle (ELN) or arrows (LTBP4)

**Table 3.** ANOVA test results comparing the scores on PC2

Comparison	P value
Congenital vs acquired	0.441
Congenital vs control	0.016
Acquired vs control	0.150

The facial shape variation associated with PC2 is shown in Figure 3. Congenital cutis laxa is associated with excessive midface lengthening and narrowing. The cutis laxa subjects

with the most dysmorphic characteristics had *ELN* mutations, which suggest that ADCL may display the most distinct craniofacial phenotype.



**Figure 4.** CVA of nose and philtrum shape

Canonical variate analysis (CVA) was applied to focus on the shape of the nose and the philtrum. As part of the CVA, a permutation test was used to examine group differences in overall mean shape. The congenital cases significantly differed from controls ( $p = 0.0006$ ); no other groups differed (Table 4). The first canonical variate (CV1) revealed congenital cutis laxa cases separating from controls and the acquired cases fell in between these two groups (Figure 4). CV1 was associated with variation in the width, length and projection of the nose and upper lip. Confirming the findings from the PCA, congenital cutis laxa was characterized by an overall lengthening and narrowing through the central midface structures as well as reduced nasal projection and upper lip protrusion.

**Table 4.** Permutation test results comparing mean shape across groups

Comparison	Procrustes distance	P value
Congenital vs acquired	0.0544	0.1334
Congenital vs control	0.0780	0.0006
Acquired vs control	0.0332	0.5868

Skin elasticity data was also analyzed to determine whether there was an association between the skin laxity and facial shape variation. The sample set of 10 (5 congenital cases and 5 acquired cases) was included in this analysis as limiting this to only congenital cases would greatly reduce the statistical power. Certain measurements were utilized such as VE, E, RT, and D and compared to the scores on PC2. The correlations were all of low to moderate magnitude; none were significantly different from zero (Table 5).

**Table 5.** Pearson correlation coefficient and comparing skin elasticity measures with scores on PC2

	Pearson Correlation	P value
PC2/D	0.338925	0.338095
PC2/RT	-0.28448	0.426479
PC2/E	-0.13388	0.714155
PC2/VE	0.399776	0.252461

Skin elasticity data was compared to the scores on CVA1 as well and the correlations were all of low to moderate magnitude with the exception of CVA1/D. None were significantly different from zero (Table 6), although the CVA1/D correlation approached significance.

**Table 6.** Pearson correlation coefficient and comparing skin elasticity measures with scores on CV1

	Pearson Correlation	P value
CVA1/D	0.629756732	0.051049
CVA1/RT	0.188894408	0.601409
CVA1/E	0.129081572	0.722462
CVA1/VE	-0.295949298	0.407974



## 4.0 DISCUSSION

There are many genetic diseases that have a distinct phenotype that an expert, having seen many patients with the same condition, can recognize just by looking at an individual's face. For example, patients with Williams syndrome have an elfin appearance. These individuals have bitemporal narrowing, a broad forehead, flattened malar region, puffy appearance around the periorbital region, wide mouth and nasal tip, long philtrum, full cheeks, and micrognathia (Ewart et al., 1993). This condition has a discernable craniofacial phenotype and has been well documented. Similarly, Marfan syndrome is a genetic disorder with a recognizable craniofacial phenotype. These patients have long faces with a high-arched palate and retrognathia (Wrestling et al., 1998; Poole, 1989). Cutis laxa, however, is less common than Marfan or Williams syndromes such that healthcare professionals rarely encounter individuals with this connective tissue disorder and therefore may have difficulty with recognizing this condition's craniofacial phenotype. As we have now demonstrated, cutis laxa does have distinct, quantifiable facial features, which will lead healthcare professionals to faster diagnosis. If enough individuals with cutis laxa are documented, physicians may be able to predict what mutation an individual has based on facial phenotype before genetic testing is initiated.

We saw excessive face lengthening and narrowing in individuals with congenital CL. Individuals with an *ELN* mutation had the most distinct facial phenotype as both individuals with greatest mid-facial difference had *ELN* mutations. The quantitative findings here confirm the

long face and bitemporal narrowing that has been qualitatively reported for the ADCL facial phenotype (Graul-Neumann et al., 2007; Hadj-Rabia et al., 2013).

Individuals with congenital CL displayed significantly less nasal projection, reduced upper lip protrusion and increased elongation of the philtrum than controls. In the literature, ADCL, ARCL1B, and ARCL1C patients have been described to have beaked, broad, and hooked noses, while ARCL3 has been described to have a short, upturned nose (Hadj- Rabia et al., 2013; Hoyer et al., 2009; Callewaert et al., 2012; Skidmore et al., 2011). Thus, the finding of reduced nasal projection is consistent with the reports in ADCL, ARCL1B, ARCL1C, and ARCL3. These individuals should have short noses (in the case of ARCL3) or noses with a drooping nasal tip (ADCL, ARCL1B, and ARCL1C).

Because the observed changes in both the overall shape of the face and the morphology of mid-facial structures were present in both pediatric and adult patients, our findings implicate CL genes in craniofacial growth and development. As all CL genes identified to date influence elastin biosynthesis (Urban et al., 2014), and in our results patients with ELN mutations showed the greatest degree of facial alterations, our study highlights a previously unappreciated role for elastin in the development and growth of the face. Elastin and fibrillin microfibrils are joined to form elastic fibers in many tissues. Therefore, it is not surprising that Marfan syndrome, caused by mutations in fibrillin-1, is characterized by an elongated face shape (de Coster et al., 2004), similar to CL. Elastic fibers are known to be present in the perichodrium and periosteum and have been postulated to inhibit the elongation of bones (Gigante et al., 1999). Initially thought to be a simple mechanical effect, it is now known that fibrillin microfibrils and elastic fibers regulate the bioavailability of transforming growth factor beta (TGF $\beta$ ) and bone morphogenetic protein (BMP) growth factors and thus regulate the proliferation and differentiation of

osteoblasts (Nistala et al., 2010), the main cell type responsible for bone formation. Unlike Marfan syndrome, CL is not characterized by dolichostenomelia and tall stature. Thus, elastin appears to regulate the growth of the cranial skeleton, but not of the long bones.

Alterations in the shape of mid-facial structures in CL also shed light onto the role of elastin in the development of the nose, lip and philtrum. Long philtrum has been observed in Williams syndrome along with other craniofacial features (Ewart et al., 1993), which, based on comparative studies on patients with partial deletions and animal models has been attributed to the deletion of *GTF2IRD1* gene, located within the Williams syndrome critical region (Tassabehji et al. 2005). Our studies now implicate elastin as a determinant of philtrum length and a likely contributor to the Williams syndrome facial phenotype.

The shape of the nose is largely determined by several cartilage bodies: the cartilage of the nasal septum, the lateral nasal, lesser alar and lower alar cartilages. These cartilages are held together by fibrous connective tissues, rich in collagen and elastic fibers (Han et al., 2004). The projection of the nose is primarily determined by the medial part (also known as medial crus) of the lower alar cartilage (Patel et al., 2013) and its ligamentous connections to the nasal septal cartilage and to the contralateral medial crus (Han et al., 2004). The relative sparsity of these ligamentous connections are thought to explain the decreased nasal projection in East Asians compared to individuals of European ancestry (Han et al., 2004). Similarly, limited nasal projection in individuals with CL may be related to the underdevelopment of nasal connective tissue.

The lack of correlation between the craniofacial shape (CVA1 and PC2) and skin elasticity measures indicates that the changes in facial shape in individuals with CL are largely unrelated to skin elasticity. However, borderline association of D, a composite variable of skin

elasticity measures and age with the greatest power to discriminate individuals with CL and controls, was observed with CV1 (p value of 0.051). CV1 captured the shape of mid-facial structures including the philtrum and the lip, composed of soft tissues conceivably influenced by skin elasticity.

A limitation of the present study is that the sample size was small; 10 participants remained after individuals were removed upon meeting exclusion criteria. However, CL is a rare disease and so it will take a longer time to obtain a larger population to study with this particular connective tissue disorder. A possible source of error is facial landmarking. This source of error was minimized by adhering to strict training and quality control criteria. A limitation of the current approach is that only the outside surface of the face was visualized. Therefore, the relative contributions of soft tissues and skeletal elements to the facial phenotype of CL remain to be determined.

Future studies should include increasing the number of participants within this data set by adding the craniofacial imaging of future CL research clinics to the existing data. This would increase the power of the analysis. Additionally, more information may be obtained about the correlation of craniofacial features and the specific gene defect responsible. This could lead to strengthened genotype-phenotype information. Other future directions may include comparing additional data to craniofacial imaging to determine new possible correlations. One example could be the DEXA data from the CL research clinic where bone density would be analyzed. If skin laxity has no bearing on the craniofacial phenotype, it would be beneficial to determine other possible factors that influence craniofacial development. Finally, the specific role for cutis laxa genes in craniofacial development could be explored through molecular biology to expand on the current study. Using an animal model such as a transgenic mouse would be ideal as CL is

the result of a dominant negative effect. This experiment could be the foundation of more in-depth quantitative analysis of the craniofacial phenotype for cutis laxa patients.

#### **4.1 PUBLIC HEALTH SIGNIFICANCE**

Rare diseases, by definition, individually affect fewer than 200,000 individuals in the US (NIH Office of Rare Diseases). However, because there are almost 7000 known rare diseases, the total number affected persons are estimated to be over 25 million nationally. Patients are often misdiagnosed or undiagnosed and suffer from early onset, chronic, debilitating conditions commonly leading to early death. In many cases, the causes of rare diseases are unknown. Pharmaceutical companies disregard rare diseases because the development of drugs is deemed uneconomical. Therefore, treatment is generally not available or inadequate. Together, these issues make rare diseases a major unaddressed public health concern. Applying state of the art 3D imaging techniques can be helpful in improving the diagnosis of rare diseases with recognizable facial characteristics, in part by training healthcare professionals (Hammond et al., 2005). Moreover, because individuals with rare Mendelian diseases are at higher risk of developing common, complex co-morbidities than the general population (Blair et al. 2013), detailed phenotyping, such as offered by 3D facial imaging is particularly justified for these conditions.

Correction of developmental defects or simple desire to improve appearance has given rise to a \$13 billion cosmetic surgery industry. The second most common cosmetic surgical intervention is rhinoplasty, the reshaping of the nose, responsible for expenditures in excess of \$1 billion in the United States (ASPS). Understanding the genetic determinants of the shape of

the nose, as in our study, may result in a more informed design and use of these surgical services, or lead to the development of biomaterials which better replicate native tissues.

Recognition of individuals by facial characteristics forms an essential element of human social interactions as illustrated by the severe social difficulties faced by individuals with prosopagnosia (Gruter et al. 2008). In addition providing individuals with information about the identity, emotional status, gaze, health and age of their counterparts, recent research suggests that facial features have profound influence on the behaviors of both the observer and the observed. Specifically, high facial width-to-height ratio in men has been associated with aggression (Carre et al., 2008), being less trustworthy (Stirrat et al., 2010) and liability to engage in deception (Haselhuhn et al., 2012). Correspondingly, individuals interacting with men of high facial width-to-height ratios behaved more selfishly (Haselhuhn et al., 2013). Objective evaluation of individuals with inherited facial dysmorphisms may help better understand persistent societal attitudes towards individuals with inherited disorders.

## **APPENDIX A: IRB APPROVAL LETTER**



## University of Pittsburgh *Institutional Review Board*

3500 Fifth Avenue  
Ground Level  
Pittsburgh, PA 15213  
(412) 383-1480  
(412) 383-1508 (fax)  
<http://www.irb.pitt.edu>

### Memorandum

To: Zsolt Urban, MD  
From: Margaret Hsieh, MD, Vice Chair  
Date: 12/20/2013  
IRB#: [MOD10020125-10](#) / PRO10020125  
Subject: Genetics of Extracellular Matrix in Health and Disease

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At its full board meeting on 12/18/2013, the University of Pittsburgh Institutional Review Board Committee reviewed and approved the requested modifications.

Please note the following information:

Modification Approval Date: 12/18/2013  
Expiration Date: 5/6/2014

For studies being conducted in UPMC facilities, no clinical activities that are impacted by the modifications can be undertaken by investigators until they have received approval from the UPMC Fiscal Review Office.

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least one month prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

**Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.**



## **APPENDIX B: CUTIS LAXA QUESTIONNAIRE**

<i>For Study Use Only:</i> Family ID: _____ Participant ID: _____
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**CUTIS LAXA**  
**Patient/Physician questionnaire**

Date of Exam: \_\_\_\_\_  
Physician Name: \_\_\_\_\_ Physician Specialty: \_\_\_\_\_  
(Please complete contact info on last page of questionnaire for both the participant and the physician.)

**RACE:**

American Indian or Alaskan ☐  
Asian ☐  
Black or African American ☐  
Native Hawaiian or Other Pacific Islander: ☐  
Other race (please specify): \_\_\_\_\_  
Two or More Races (please specify): \_\_\_\_\_  
White: ☐

**ETHNICITY:**

Hispanic or Latino ☐  
Other ethnicity (please specify): \_\_\_\_\_

**Definitions:**

**American Indian or Alaska Native:** A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliation or community attachment.  
**Asian:** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.  
**Black or African American:** A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."  
**Native Hawaiian or Other Pacific Islander:** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.  
**White:** A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.  
**Hispanic or Latino:** A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," can be used in addition to "Hispanic or Latino".

## **PAST MEDICAL HISTORY**

### **Birth Data:**

Date of birth (mo/day/year): \_\_\_\_/\_\_\_\_/\_\_\_\_

Male: ☐ Female: ☐

Gestational age at birth: \_\_\_\_\_ weeks

Birth weight: \_\_\_\_\_ Length: \_\_\_\_\_ Head circumference: \_\_\_\_\_

Amniotic fluid: Normal: ☐ Oligohydramnios: ☐ Polyhydramnios: ☐

Fetal movements: Normal: ☐ Reduced: ☐ Increased: ☐

Fetal ultrasound: Normal: ☐ Abnormal: ☐ (see table) Not Performed: ☐

<b>Ultrasound Abnormalities:</b>	<b>Gestational Age:</b>

### **Death Data:**

If deceased, date of death (mo/day/year): \_\_\_\_/\_\_\_\_/\_\_\_\_

Cause of death:

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### **Previous Surgical History:**

History of abdominal vascular surgery: Yes ☐ No ☐ ? ☐

History of any facial surgeries: Yes ☐ No ☐ ? ☐

If yes, type(s) of surgeries:

**Cosmetic surgeries:**

Face lift:    Yes ☐    No ☐    Don't Know ☐    Date(s): \_\_\_\_\_

Other: \_\_\_\_\_ Date(s): \_\_\_\_\_

\_\_\_\_\_ Date(s): \_\_\_\_\_

**Previous Hospitalizations:**

History of any trauma that affected the facial structure:    Yes ☐    No ☐    Don't Know ☐

If yes, document here:

**Medications:**

**Allergies:**    Yes ☐    No ☐    Don't Know ☐

Allergens:

Skin affected by allergies:    Yes ☐    No ☐    Don't Know ☐

### 3 GENERATION PEDIGREE

Patient: \_\_\_\_\_  
 Historian: \_\_\_\_\_  
 Completed By: \_\_\_\_\_ Date: \_\_\_\_\_  
 Updated By: \_\_\_\_\_ Date: \_\_\_\_\_

Paternal Ethnicity: \_\_\_\_\_  
 Maternal Ethnicity: \_\_\_\_\_  
 Consanguinity: \_\_\_\_\_

#### Family Review of Systems (Please note findings on pedigree):

LD/DD/MR	Yes	No	Gastrointestinal Problems	Yes	No	Diverticulac	Yes	No
Respiratory Problems	Yes	No	Genitourinary Problems	Yes	No	Hernias	Yes	No
Cardiovascular Problems	Yes	No	Skeletal Problems	Yes	No	Growth Delay	Yes	No
Skin: At least 2-3 of the following							Yes	No
(i) Loose, lax; (ii) Redundant; (iii) Inelastic; (iv) Pre-mature aging of the skin; (v) Skin wrinkling								

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## CLINICAL DESCRIPTION

Age at examination: \_\_\_\_\_

Age when symptoms began: \_\_\_\_\_

### **SKIN:**

Lax: Yes ☐ No ☐ ? ☐

Redundant: Yes ☐ No ☐ ? ☐

Inelastic: Yes ☐ No ☐ ? ☐

Loose facial skin: Yes ☐ No ☐ ? ☐

Premature aging of skin: Yes ☐ No ☐ ? ☐

Skin wrinkling: Yes ☐ No ☐ ? ☐

Affected skin: Generalized ☐ Localized ☐

If localized: Symmetric ☐ Asymmetric ☐ Flexural ☐ Focal ☐

Transparent skin: Yes ☐ No ☐ ? ☐

Inflammatory skin disease: Yes ☐ No ☐ ? ☐

Alopecia: Yes ☐ No ☐ ? ☐ Location: \_\_\_\_\_

Other skin findings: \_\_\_\_\_

### **CRANIOFACIAL:**

Facial drooping: Yes ☐ No ☐ ? ☐

Wizened appearance: Yes ☐ No ☐ ? ☐

Large fontanel: Yes ☐ No ☐ ? ☐

Delayed closure of fontanel: Yes ☐ No ☐ ? ☐

Oxycephaly: Yes ☐ No ☐ ? ☐

Macrocephaly: Yes ☐ No ☐ ? ☐ OFC: \_\_\_\_\_

Microcephaly: Yes ☐ No ☐ ? ☐ OFC %ile: \_\_\_\_\_

Frontal bossing: Yes ☐ No ☐ ? ☐

Reversed-V eyebrows: Yes ☐ No ☐ ? ☐

Downslanting palpebral fissures: Yes ☐ No ☐ ? ☐

Hooked nose: Yes ☐ No ☐ ? ☐

Long philtrum: Yes ☐ No ☐ ? ☐ Length: \_\_\_\_\_ (cm)

Retrognathia/Micrognathia: Yes ☐ No ☐ ? ☐ (If Yes, circle which type)

Occipital horns: Yes ☐ No ☐ ? ☐

Other craniofacial findings: \_\_\_\_\_

**ORAL:**Vocal cord laxity: Yes ☐ No ☐ ? ☐Hoarse voice: Yes ☐ No ☐ ? ☐Dental caries: Yes ☐ No ☐ ? ☐

Details: \_\_\_\_\_

Cleft palate Yes ☐ No ☐ ? ☐

Details/type: \_\_\_\_\_

Bifid uvula Yes ☐ No ☐ ? ☐Other oral findings:**EYES/EARS:**Retinovascular tortuosity: Yes ☐ No ☐ ? ☐Corneal opacities: Yes ☐ No ☐ ? ☐Corneal arcus: Yes ☐ No ☐ ? ☐Cataract: Yes ☐ No ☐ ? ☐Myopia: Yes ☐ No ☐ ? ☐Blindness: Yes ☐ No ☐ ? ☐Hearing Loss: Yes ☐ No ☐ ? ☐ dB loss (left): \_\_\_\_\_Deafness: Yes ☐ No ☐ ? ☐ dB loss (right): \_\_\_\_\_Other eye findings:**NEUROBEHAVIORAL:**Deep tendon reflexes: Present ☐ Absent ☐ Not Checked ☐Hypotonia: Yes ☐ No ☐ ? ☐Cong. bilateral athetosis: Yes ☐ No ☐ ? ☐Seizures: Yes ☐ No ☐ ? ☐ Frequency: \_\_\_\_\_Autism: Yes ☐ No ☐ ? ☐ Details: \_\_\_\_\_Mental retardation: Yes ☐ No ☐ ? ☐ IQ Test: \_\_\_\_\_ Score: \_\_\_\_\_

Education history:

Social history:

Other neurobehavioral findings:**RESPIRATORY:**Bronchiectasis: Yes ☐ No ☐ ? ☐Hypoplastic lungs (newborn): Yes ☐ No ☐ ? ☐Emphysema: Yes ☐ No ☐ ? ☐

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Tachypnea: Yes ☐ No ☐ ? ☐  
Pneumonia: Yes ☐ No ☐ ? ☐ Frequency: \_\_\_\_\_  
Asthma: Yes ☐ No ☐ ? ☐ Type: \_\_\_\_\_  
Chest CT findings and date: \_\_\_\_\_  
Pulmonary function test (PFT) findings and date: \_\_\_\_\_  
Current smoker: Yes ☐ No ☐  
Cigarette packs per day: \_\_\_\_\_  
Years smoked: \_\_\_\_\_  
Other respiratory findings: \_\_\_\_\_

### **CARDIOVASCULAR:**

Cor pulmonale: Yes ☐ No ☐ ? ☐  
Right ventricular hypertrophy: Yes ☐ No ☐ ? ☐  
Infundibular stenosis: Yes ☐ No ☐ ? ☐  
Pulmonary valve stenosis: Yes ☐ No ☐ ? ☐  
Pulmonary artery stenosis: Yes ☐ No ☐ ? ☐  
Murmur (6 scale): \_\_\_\_\_  
Pressure gradient (Hgmm): \_\_\_\_\_  
Peak velocity (m/s): \_\_\_\_\_  
Peripheral pulmonary stenosis: Yes ☐ No ☐ ? ☐  
Supravalvular aortic stenosis: Yes ☐ No ☐ ? ☐  
Valvular aortic stenosis: Yes ☐ No ☐ ? ☐  
Murmur (6 scale): \_\_\_\_\_  
Pressure gradient (Hgmm): \_\_\_\_\_  
Peak velocity (m/s): \_\_\_\_\_  
Aortic dilatation: Yes ☐ No ☐ ? ☐  
Level: \_\_\_\_\_ Diameter: \_\_\_\_\_  
Arterial tortuosity: Yes ☐ No ☐ ? ☐  
Which arteries: \_\_\_\_\_  
Arterial aneurysms: Yes ☐ No ☐ ? ☐  
Which arteries: \_\_\_\_\_  
Raynaud's phenomenon: Yes ☐ No ☐ ? ☐  
Venous varicosity: Yes ☐ No ☐ ? ☐  
Echocardiogram (Echo) findings and date: \_\_\_\_\_  
Other cardiovascular findings: \_\_\_\_\_

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**GASTROINTESTINAL:**

Diverticulæ: Esophageal: Yes ☐ No ☐ ? ☐  
 Intestinal: Yes ☐ No ☐ ? ☐  
 Rectal: Yes ☐ No ☐ ? ☐  
 Other: \_\_\_\_\_

Other gastrointestinal findings: \_\_\_\_\_

**GENITOURINARY:**

Bladder diverticulæ: Yes ☐ No ☐ ? ☐  
 Genital prolapse: Yes ☐ No ☐ ? ☐  
 Obstructive uropathy: Yes ☐ No ☐ ? ☐  
 Bladder neck obstruction: Yes ☐ No ☐ ? ☐

Other genitourinary findings: \_\_\_\_\_

**MUSCULOSKELETAL:**

Hernias: Inguinal: Yes ☐ No ☐ ? ☐  
 Diaphragmatic: Yes ☐ No ☐ ? ☐  
 Hiatal: Yes ☐ No ☐ ? ☐  
 Umbilical: Yes ☐ No ☐ ? ☐  
 Hip dislocation: Yes ☐ No ☐ ? ☐  
 Thumbs and/or toes dislocated: Yes ☐ No ☐ ? ☐  
 Other dislocations/subluxations: Yes ☐ No ☐ ? ☐ Details: \_\_\_\_\_  
 \_\_\_\_\_  
 Joint pain: Yes ☐ No ☐ ? ☐  
 Joint laxity: Yes ☐ No ☐ ? ☐  
 Fractures: Yes ☐ No ☐ ? ☐ #/Site(s): \_\_\_\_\_  
 Osteopenia/Osteoporosis: Yes ☐ No ☐ ? ☐ Hip? ☐ Spine? ☐ Total? ☐  
 Pectus carinatum: Yes ☐ No ☐ ? ☐  
 Pectus excavatum: Yes ☐ No ☐ ? ☐  
 Scoliosis: Yes ☐ No ☐ ? ☐ Degree: \_\_\_\_\_  
 Flat feet: Yes ☐ No ☐ ? ☐  
 Short broad clavicles: Yes ☐ No ☐ ? ☐  
 Fused carpal bones: Yes ☐ No ☐ ? ☐

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Other musculoskeletal findings:

**GROWTH:**

Growth deficiency/delay:

Prenatal: Yes ☐ No ☐ ? ☐

Postnatal Yes ☐ No ☐ ? ☐

Dwarfism Yes ☐ No ☐ ? ☐

Physical data:

Age	Weight	Height	Head Circumference

**OTHER ABNORMALITIES NOT LISTED ABOVE:**

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**LABORATORY:**

Abnormal elastic fibers:

Histology: Yes ☐ No ☐ ? ☐

EM: Yes ☐ No ☐ ? ☐

Glycosylation Testing: Yes ☐ No ☐ ? ☐ Result: \_\_\_\_\_

Genetic testing: Yes ☐ No ☐ ? ☐ Details/Result: \_\_\_\_\_

Serum ceruloplasmin: \_\_\_\_\_

Serum copper: \_\_\_\_\_

**ADDITIONAL INFORMATION:**

Clinical photographs available: Yes ☐ No ☐

DNA available: Yes ☐ No ☐

Cultured cells available: Yes ☐ No ☐

Pathology slides available: Yes ☐ No ☐

Frozen tissue available: Yes ☐ No ☐

Imaging Studies: Yes ☐ No ☐ Details: \_\_\_\_\_

Patient available for re-examination: Yes ☐ No ☐

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**CUTIS LAXA**  
**Contact Information**

**PARTICIPANT:**

Family ID: \_\_\_\_\_ (to be filled in by study coordinator)

Participant ID: \_\_\_\_\_ (to be filled in by study coordinator)

Participant's Name: \_\_\_\_\_

Parent's or Guardian's Name (if participant is minor): \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

Home Phone: \_\_\_\_\_ Cell Phone: \_\_\_\_\_

Fax: \_\_\_\_\_

**EMAIL:** \_\_\_\_\_

**REFERRING PHYSICIAN:**

Physician's Name: \_\_\_\_\_

Physician Address: \_\_\_\_\_

\_\_\_\_\_

Phone: \_\_\_\_\_

Fax: \_\_\_\_\_

**EMAIL:** \_\_\_\_\_

## BIBLIOGRAPHY

- Aldridge, K., Boyadjiev, S.A., Capone, G.T., DeLeon, V.B., Richtsmeier, J.T. (2005) Precision and Error of Three-Dimensional Phenotypic Measures Acquired From 3dMD Photogrammetric Images. *American Journal of Medical Genetics* 138A, 247-253.
- The American Society of Plastic Surgeons. <http://www.plasticsurgery.org/Documents/news-resources/statistics/2013-statistics/cosmetic-procedures-average-fees.pdf>
- Artopoulos, A., Buytaert, J.A.N., Dirckx, J.J.J., Coward, T.J. (2013) Comparison of the accuracy of digital stereophotogrammetry and projection moiré profilometry for three dimensional imaging of the face. *International Journal of Oral and Maxillofacial Surgery* <http://dx.doi.org/10.1016/j.ijom.2013.10.005>.
- Basel-Vanagaite, L. et al. (2009) RIN2 Deficiency Results in Macrocephaly, Alopecia, Cutis Laxa, and Scoliosis: MACS Syndrome. *The American Journal of Human Genetics* 85, 254-263.
- Berk, D.R. et al. (2012) Cutis laxa: a review. *Journal of the American Academy of Dermatology* 66(5), 842.e1-17.
- Bicknell, L.S. et al. (2008) A missense mutation in ALDH18A1, encoding D1-pyrroline-5-carboxylate synthase (P5CS), causes an autosomal recessive neurocutaneous syndrome. *European Journal of Human Genetics* 16, 1176-1186.
- Blair, D.R. et al. (2013) A nondegenerate code of deleterious variants in Mendelian loci contributes to complex disease risk. *Cell* 155(1), 70-80.
- Callewaert, B. et al. (2011) New Insights into the Pathogenesis of Autosomal-Dominant Cutis Laxa with Report of Five ELN Mutations. *Human Mutation* 32(4), 445-55.
- Callewaert, B. et al. (2012) Comprehensive clinical and molecular analysis of 12 families with type 1 recessive cutis laxa. *Human Mutation* 34(1), 111-121.
- Callewaert, B.L. et al. (2008) Arterial Tortuosity Syndrome: Clinical and Molecular Findings in 12 Newly Identified Families. *Human Mutation* 29(1), 150-158.

- Carre, J.M., McCormick, C.M. (2008) In your face: facial metrics predict aggressive behaviour in the laboratory and in varsity and professional hockey players. *Proceedings. Biological society/The Royal Society* 275(1651), 2651-6.
- Castori, M. et al. (2012) Adult presentation of arterial tortuosity syndrome in a 51-year-old woman with a novel homozygous c.1411p1G>A mutation in the SLC2A10 gene. *American Journal of Medical Genetics* 158, 1164-1169.
- Cistulli, P.A., Gotsopoulos, H., Sullivan, C.E. (2001) Relationship between craniofacial abnormalities and sleep-disordered breathing in Marfan's syndrome. *Chest* 5, 1455-60.
- Claus, S. et al. (2008) A p.C217R Mutation in Fibulin-5 from Cutis Laxa Patients Is Associated with Incomplete Extracellular Matrix Formation in a Skin Equivalent Model. *Journal of Investigative Dermatology* 128, 1442-1450.
- Coucke, P.J. et al. (2006) Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. *Nature Genetics* 38(4), 452-7.
- Das, S. et al. (1995) Similar Splicing Mutations of the Menkes/Mottled Copper-Transporting ATPase Gene in Occipital Horn Syndrome and the Blotchy Mouse. *American Journal of Human Genetics* 56, 570-576.
- Dasouki, M. et al. (2007) Compound Heterozygous Mutations in Fibulin-4 Causing Neonatal Lethal Pulmonary Artery Occlusion, Aortic Aneurysm, Arachnodactyly, and Mild Cutis Laxa. *American Journal of Medical Genetics Part A* 143A:2635–2641.
- De Coster, P., De Pauw, G., Martens, L., De Paepe, A. (2004) Craniofacial Structure in Marfan Syndrome: A Cephalometric Study. *American Journal of Medical Genetics* 131A:240–248.
- De Coster, P.J., Martens, L.C., De Paepe, A. (2004) Orofacial manifestations of congenital fibrillin deficiency: pathogenesis and clinical diagnostics. *Pediatric dentistry* 26(6), 535-7.
- Dimopoulou, A. et al. (2013) Genotype–phenotype spectrum of PYCR1-related autosomal recessive cutis laxa. *Molecular Genetics and Metabolism* 110, 352-361.
- Erickson, L.K., Opitz, J.M., Zhou, H. (2012) Lethal Osteogenesis Imperfecta–Like Condition with Cutis Laxa and Arterial Tortuosity in MZ Twins Due to a Homozygous Fibulin-4 Mutation. *Pediatric and Developmental Pathology* 15(2), 137-141.
- Ewart, A.K. et al. (1993) Hemizygoty at the elastin locus in a developmental disorder, Williams syndrome. *Nature Genetics* 5, 11-16.
- Farkas, LG. *Anthropometry of the head and face*. 2nd ed. New York: Raven Press, 1994.
- Fischer, B. et al. (2012) Further characterization of ATP6V0A2-related autosomal recessive cutis laxa. *Human Genetics* 131(11), 1761-1773.

- Gigante, A., Chillemi, C., Greco, F. (1999) Changes of elastic fibers in musculoskeletal tissues of Marfan syndrome: a possible mechanism of joint laxity and skeletal overgrowth. *Journal of Pediatric Orthopedics* 19(3), 283-8.
- Graul-Neumann, L.M. et al. (2008) Highly Variable Cutis Laxa Resulting From a Dominant Splicing Mutation of the Elastin Gene. *American Journal of Medical Genetics Part A* 146A, 977-983.
- Grewe, J., Jorgenson, R., McKusick, V. (1971) Model system of craniofacial growth: Marfan's syndrome. *Journal of Dental Research* 50(6), 1501-2.
- Gruter, T., Gruter, M., Carbon, C.C. (2008) Neural and genetic foundations of face recognition and prosopagnosia. *Journal of Neuropsychology* 2(Pt 1), 79-97.
- Guernsey, D.L. et al. (2009) Mutation in Pyrroline-5-Carboxylate Reductase 1 Gene in Families with Cutis Laxa Type 2. *American Journal of Human Genetics* 85, 120-129.
- Hadj-Rabia, S. et al. (2013) Twenty patients including 7 probands with autosomal dominant cutis laxa confirm clinical and molecular homogeneity. *Orphanet Journal of Rare Diseases* 8(1), 36.
- Hammond, P. et al. (2005) Discriminating power of localized three-dimensional facial morphology. *American Journal of Human Genetics* 77, 999-1010.
- Han, S.K., Lee, D.G., Kim, J.B., Kim, W.K. (2004) An anatomic study of nasal tip supporting structures. *Annals of Plastic Surgery* 52(2), 134-9.
- Haselhuhn, M.P., Wong, E.M. (2012) Bad to the bone: facial structure predicts unethical behaviour. *Proceedings. Biological sciences/The Royal Society* 279(1728), 571-6.
- Haselhuhn, M.P., Wong, E.M., Ormiston, M.E. (2013) Self-fulfilling prophecies as a link between men's facial width-to-height ratio and behavior. *PLoS One* 8(8), e72259. doi: 10.1371.
- He, D. et al. (2012) Polymorphisms in the Human Tropoelastin Gene Modify In Vitro Self-Assembly and Mechanical Properties of Elastin-Like Polypeptides. *PLoS One* 7(9), e46130.
- Heike, C.L., Cunningham, M.L., Hing, A.V., Stuhau, E., Starr, J.R. (2009) Picture Perfect? Reliability of Craniofacial Anthropometry Using Three-Dimensional Digital Stereophotogrammetry. *Plastic and Reconstructive Surgery* 124(4), 1261-1272.
- Hoyer, J. et al. (2009) Lethal cutis laxa with contractural arachnodactyly, overgrowth and soft tissue bleeding due to a novel homozygous fibulin-4 gene mutation. *Clinical Genetics* 76, 276-281.
- Hu, Q. et al. (2006) Fibulin-5 mutations: mechanisms of impaired elastic fiber formation in recessive cutis laxa. *Human Molecular Genetics* 15(23), 3379-86.

- Huchtagowder, V. et al. (2006) Fibulin-4: A Novel Gene for an Autosomal Recessive Cutis Laxa Syndrome. *The American Journal of Human Genetics* 78, 1075-1080.
- Huchtagowder, V. et al. (2009) Loss-of-function mutations in ATP6V0A2 impair vesicular trafficking, tropoelastin secretion and cell survival. *Human Molecular Genetics* 18 (12), 2149-2165.
- Kainulainen, K., Pulkkinen, L., Savolainen, A., Kaitila, I., Peltonen, L. (1990) Location on chromosome 15 of the gene defect causing Marfan syndrome. *New England Journal of Medicine* 323, 935-939.
- Kaler, S.G. et al. (1994) Occipital horn syndrome and a mild Menkes phenotype associated with splice site mutations at the MNK locus. *Nature Genetics* 8, 195-202.
- Kaplanayil, M. et al. (2012) Characterization of a distinct lethal arteriopathy syndrome in twenty-two infants associated with an identical, novel mutation in FBLN4 gene, confirms fibulin-4 as a critical determinant of human vascular elastogenesis. *Orphanet journal of rare diseases* 7, 61.
- Kennerson, M.L. et al. (2010) Missense mutations in the copper transporter gene ATP7A cause x-linked distal hereditary motor neuropathy. *American Journal of Human Genetics* 86(3), 343-352.
- Kornak, U. et al. (2008) Impaired glycosylation and cutis laxa caused by mutations in the vesicular H<sup>+</sup>-ATPase subunit ATP6V0A2. *Nature Genetics* 40(1), 32-34.
- Kouwenberg, D. et al. (2011) Recognizable Phenotype With Common Occurrence of Microcephaly, Psychomotor Retardation, but no Spontaneous Bone Fractures in Autosomal Recessive Cutis Laxa Type IIB Due to PYCR1 Mutations. *American Journal of Medical Genetics* 155, 2331-2332.
- Kowal, R.C., Richardson, J.A., Miano, J.M., Olson, E.N. (1999) EVEC, a novel epidermal growth factor-like repeat-containing protein upregulated in embryonic and diseased adult vasculature. *Circulation Research* 84(10), 1166-76.
- Lin, D. et al. (2011) A Novel Mutation in PYCR1 Causes an Autosomal Recessive Cutis Laxa With Premature Aging Features in a Family. *American Journal of Medical Genetics* 155, 1285-1289.
- Loeys, B. et al. (2002) Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. *Human Molecular Genetics* 11(18), 2113-2118.
- Loskin, A. et al. (2005) An Objective Evaluation of Breast Symmetry and Shape Differences Using 3-Dimensional Images. *Annals of Plastic Surgery* 55(6), 571-5.
- Maal, T.J.J. et al. (2010) Registration of 3-Dimensional Facial Photographs for Clinical Use. *Journal of Oral and Maxillofacial Surgery* 68(10), 2391-2401.

- Motohashi, N. (1985) Craniofacial dysmorphology in syndromes associated with abnormal physical growth. *Journal of Craniofacial Genetics and Developmental Biology* 1, 211-225.
- Nistala, H. et al. (2010) Fibrillin-1 and -2 differentially modulate endogenous TGF- $\beta$  and BMP bioavailability during bone formation. *The Journal of Cell Biology* 190(6), 1107-21.
- Patel, K.B., Mendonca, D.A., Skolnick, G., Woo, A.S. (2013) Anatomical study of the medial crura and the effect on nasal tip projection in open rhinoplasty. *Plastic and reconstructive surgery* 132(4), 787-93.
- Poole, A.E. Craniofacial aspects of the Marfan syndrome. *Birth defects original article series* 25(4), 73-81.
- Renard, M. et al. (2010) Altered TGF $\beta$  signaling and cardiovascular manifestations in patients with autosomal recessive cutis laxa type I caused by fibulin-4 deficiency. *European Journal of Human Genetics* 18, 895-901.
- Reversade, B. et al. (2009) Mutations in PYCR1 cause cutis laxa with progeroid features. *Nature Genetics* 41(9), 1016-1021.
- Sawyer, S.L. et al. (2012) Longer Term Survival of a Child With Autosomal Recessive Cutis Laxa Due to a Mutation in FBLN4. *American Journal of Medical Genetics Part A* 161A:1148–1153.
- Skidmore, D.L. et al. (2011) Further expansion of the phenotypic spectrum associated with mutations in ALDH18A1, encoding Delta-Pyrroline-5-Carboxylate Synthase (P5CS). *American Journal of Medical Genetics* 155, 1848-1856.
- Sood, S., Eldadah, Z.A., Krause, W.L., McIntosh, I., Dietz, H.C. (1996) Mutation in fibrillin-1 and the Marfanoid craniosynostosis (Shprintzen-Goldberg syndrome). *Nature* 382, 209-211.
- Stirrat, M., Perrett, D.I. (2010) Valid facial cues to cooperation and trust: male facial width and trustworthiness. *Psychological science* 21(3), 349-54.
- Syx, D. et al. (2010) The RIN2 syndrome: a new autosomal recessive connective tissue disorder caused by deficiency of Ras and Rab interactor 2 (RIN2). *Human Genetics* 128, 79-88.
- Takahashi, Y. et al. (2013) Artery Tortuosity Syndrome Exhibiting Early-Onset Emphysema With Novel Compound Heterozygous SLC2A10 Mutations. *American Journal of Medical Genetics Part A* 161A, 856–859.
- Tassabehji, M. et al. (1998) An elastin gene mutation producing abnormal tropoelastin and abnormal elastic fibres in a patient with autosomal dominant cutis laxa. *Human Molecular Genetics* 7(6), 1021-8.



- Tassabehji, M. et al. (2005) GTF2IRD1 in Craniofacial Development of Humans and Mice. *Science* 310, 1184-1187.
- Tumer, Z. (2013) An Overview and Update of ATP7A Mutations Leading to Menkes Disease and Occipital Horn Syndrome. *Human Mutation* 34(3), 417-29.
- Uitto, J., Qiaoli L., Urban, Z. (2012) The complexity of elastic fibre biogenesis in the skin – a perspective to the clinical heterogeneity of cutis laxa. *Experimental Dermatology* 22(2):88-92.
- Urban, Z. et al. (2009) Mutations in LTBP4 Cause a Syndrome of Impaired Pulmonary, Gastrointestinal, Genitourinary, Musculoskeletal, and Dermal Development. *The American Journal of Human Genetics* 85, 593-605.
- Urban, Z., Davis, E.C. (2014) Cutis laxa: intersection of elastic fiber biogenesis, TGF $\beta$  signaling, the secretory pathway and metabolism. *Matrix biology: journal of International Society for Matrix biology* 33, 16-22.
- Weinberg, S.M. et al. (2004) Digital three-dimensional photogrammetry: evaluation of anthropometric precision and accuracy using a Genex 3D camera system. *The Cleft Palate-Craniofacial Journal* 41(5), 507-18.
- Weinberg, S.M. et al. (2006) Anthropometric Precision and Accuracy of Digital Three-Dimensional Photogrammetry: Comparing the Genex and 3dMD Imaging Systems with One Another and with Direct Anthropometry. *The Journal of Craniofacial Surgery* 17(3), 477-83.
- Wong, J.Y. et al. (2008) Validity and reliability of craniofacial anthropo-metric measurement of 3d digital photogrammetric images. *The Cleft Palate-Craniofacial Journal* 45(3), 232–239.
- Wrestling, L., Mohlin, B., Bresin, A. (1998) Craniofacial manifestations in the Marfan syndrome: palatal dimensions and a cephalometric analysis. *Journal of craniofacial genetics and developmental biology* 18(4), 211-8.
- Yanagisawa, H. et al. (2002) Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* 415(6868), 168-71.
- Yildirim, Y., Tolun, A., Tuysuz, B. (2010) The Phenotype Caused by PYCR1 Mutations Corresponds to GeroDERMA Osteodysplasticum Rather than Autosomal Recessive Cutis Laxa Type 2. *American Journal of Medical Genetics* 155, 134-140.
- Zaidi, S.H.E. et al. (2009) Congenital diaphragmatic abnormalities in arterial tortuosity syndrome patients who carry mutations in the SLC2A10 gene. *Clinical Genetics* 75, 588–589.
- Zhang, M.C. et al. (1999) Cutis laxa arising from frameshift mutations in exon 30 of the elastin gene (ELN). *The Journal of Biological Chemistry* 274(2), 981-6.